Structure

In This Issue

Encapsulating Membrane-Anchored Proteins

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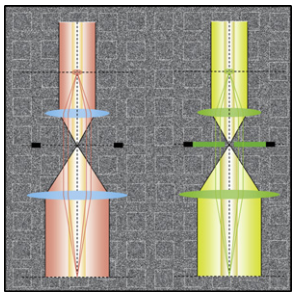
Perhaps 5%–10% of proteins bind to the membranes via a covalently attached lipid. High-resolution information about the nature of lipidated proteins is remarkably sparse, often because of solubility problems caused by the exposed fatty acids. Reverse micelle encapsulation is used here by Valentine et al. to study two myristoylated proteins in their lipid-extruded states: myristoylated recoverin, which is a switch in the Ca^{2+} signaling pathway in vision, and the myristoylated HIV-1 matrix protein. The approach seems broadly applicable to this class of membrane-anchored proteins that have eluded structural characterization by conventional approaches.

Asymmetric Protein Complexes Served on a Zernike Phase Plate



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Cryo-EM imaging of proteins less than a megadalton is faced with challenges due to low contrast. There is an increased interest in improving the phase-contrast by various approaches such Zernike-phase-contrast (ZPC) cryo-EM. Yet, how single particle cryo-EM would benefit from ZPC remains unclear. Here, Chang et al. demonstrate that ZPC improves the 3D-EM workflow by better image characterization and classification, and significant reduction in the number of particles (100–500 kDa) required for 3D reconstruction to near atomic resolution.

APOBEC3G Catalytic Domain Keen on Oligomerization

PAGE 28

APOBEC3G is a DNA cytidine deaminase that has antiviral activity against HIV-1 and other pathogenic viruses. A crystal structure of the catalytically active C-terminal domain was determined to 2.25 Å by Shandilya et al., here. This structure and complementary mutagenesis validates structural features previously observed in NMR studies. Oligomerization is postulated to be critical for the function of APOBEC3G. Intermolecular interfaces observed in this crystal structure, some containing residues critical to both APOBEC3G's DNA deaminase and anti-HIV activity as well as a unique zinc binding site, suggest potential models for APOBEC3 G oligomerization.

May the Single Force Be with You

PAGE 39

Mitochondrial ADP/ATP carriers are inhibited by two natural compounds, atractyloside (ATR) or carboxy-atractyloside (CATR), which differ by one carboxylate group. Kedrov et al. examined the interactions of the inhibitors with the carrier by single-molecule force spectroscopy. Transmembrane α helices of the ATR-inhibited carrier displayed heterogeneous mechanical and kinetic properties. In contrast to ATR, CATR-binding substantially increased the kinetic stability of α helix H2 and tuned the mechanical flexibility of α helices H5 and H6. The tight docking of the inhibitor, together with a reduction in the flexibility of α helix H2 leads to efficient inhibition of the ADP/ATP carrier and enhanced affinity of the complex.

Life of a Gammaherpesvirus

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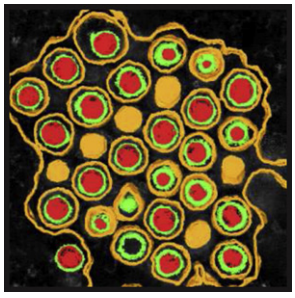


What are the stages of a viral life cycle and is it possible to visualize them at high resolution? A study by Peng et al. now provides some answers to this question by presenting a three-dimensional (3D) visualization of the life cycle of a gammaherpesvirus in host cells. Unprecedented 3D details of viral and cellular structures at different stages of the viral life cycle were observed. In particular, viral DNA injection into the nucleus from incoming capsids docking at nuclear pore complexes, DNA encapsidation in progeny capsids, and nuclear-membrane invaginations associated with nuclear egress were captured. These 3D observations not only provide the structural basis for understanding virus-host interactions during gammaherpesvirus infection, but also demonstrate the power of electron tomography in dissecting complex cellular processes.

Opportunistic Pathogen with a Sweet Tooth

PAGE 59

Pathogenic bacteria often use host glycoconjugates as receptors for recognition and adhesion. Bacterial toxin, adhesions, or soluble lectins have been demonstrated to be involved in binding to such glycans. The opportunistic pathogen *Burkholderia cenocepacia* expresses several soluble lectins, including BC2L-C, and is investigated here by Šulák et al. The C terminus domain is very similar to the recently described calcium-dependent mannose-binding lectin BC2L-A. The N terminus domain binds to fucosylated human histo-blood group epitopes H-type 1, Lewis b, and Lewis. Its structure reveals a new lectin family adopting a trimetric jelly roll arrangement with striking similarity to TNF-like proteins. BC2L-C is therefore a super-lectin with dual specificity.

G-Quadruplex Scaffold from Human Intronic Sequence

PAGE 73

Kuryavyi and Patel describe an NMR-based solution structure of a four-stranded DNA G-quadruplex adopted by a guanine-rich segment within the 5́-intron of the human chl1 gene. The folding topology contains two unique features involving positioning of the first guanine and a V-shaped loop reported here for the first time for a unimolecular G-quadruplex. Unexpectedly, the structural arrangement of guanine bases within the chl1 intronic DNA G-quadruplex bears striking analogy to the structure of the group I intron RNA catalytic site. The authors speculate that such a DNA G-quadruplex scaffold could provide a platform for trans-etherification catalysis leading to recombination of genetic information.

Mechanism of the DNA polymerase Translocation

PAGE 83

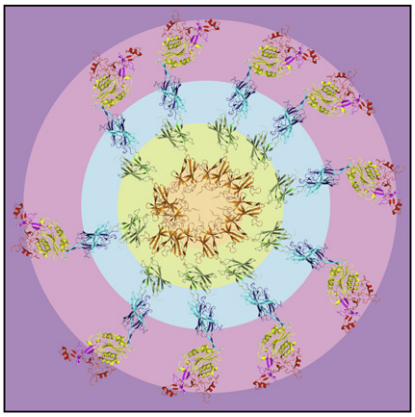
High-fidelity DNA polymerases copy DNA rapidly and accurately by adding deoxynucleotide triphosphates to a growing primer strand. An essential step following nucleotide addition is the translocation of the DNA substrate by one base pair. Golosov et al. use molecular dynamics simulations to show that the translocation mechanism involves a series of conformational changes that start with a polymerase "fingers-opening" transition. This enables the DNA displacement and the insertion of the template base into the preinsertion site. Release of the pyrophosphate appears to be the trigger for the opening transition, and the authors suggest that a corresponding mechanism is applicable to other polymerases.

Dehydratase Domains from the Curacin Polyketide Biosynthetic Pathway

PAGE 94

Polyketide synthases (PKS) are enzymes responsible for biosynthesis of large number of natural products via an assembly line of multidomain modules. A type of enzymatic module in the assembly line is dehydratase domain which catalyzes formation of an α,β-double bond in the nascent polyketide intermediate. Structures of all dehydratase (DH) domains from the Curacin A biosynthetic pathway, described by Akey et al., reveal an active site located at a sharp bend within a substrate tunnel, which restricts the conformation of the double bonded product. Structural elements covering the active site are likely mobile, suggesting a general mechanism with which a large variety of conformationally restricted and/or bulky intermediates may easily access and exit the active site.

How Pilus Adhesin Knows Its Target



PAGE 106

Pili are hair-like appendages exposed on the surface of pathogenic bacteria that play important roles in infection; they often carry adhesins, proteins that are essential for recognition of target cells, on the tips of their elongated structures. In this work, Izoré et al. used X-ray crystallography to decipher a structure of RrgA, the major adhesin of the pilus of the human pathogen *Streptococcus pneumoniae*, causative agent of meningitis, bacteremia, and pneumonia. The structure reveals that RrgA carries domains that are capable of recognizing sugars as well as elements of the extracellular matrix, and suggests how pneumococci are able to efficiently adhere to target cells.

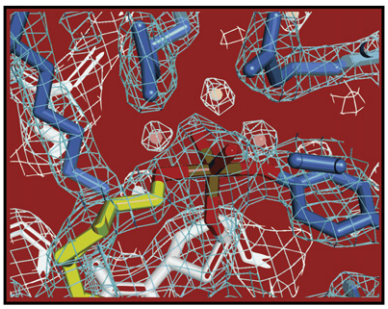
Calcium-Myristoyl Switch: Not a General Mechanism

PAGE 116

It was proposed that neuronal calcium-binding proteins or NCS proteins undergo "calcium-myristoyl switch" upon Ca^{2+} binding. The current study by Orban et al., together with crystallographic data previously published in *Structure*, demonstrate that this mechanism is rather an exception for one family member, recoverin, and not general to the NCS family. Specifically, in the current study, the authors use hydrogen-deuterium exchange and radiolytic labeling, both coupled to mass spectrometry, and molecular dynamics simulations of GCAP1 and myr-GCAP1 in the Ca^{2+}-free and Ca^{2+}-bound forms to study conformational changes induced by the presence of the myristoyl group and Ca^{2+} to better understand the activation process mediated by GCAP.

Topoisomerase Mechanism of Catalysis

PAGE 127



Type IB topoisomerases are enzymes that relax DNA that is either over- or under wound. The smallpox virus encodes its own type IB topoisomerase that facilitates transcription of its genes. Perry et al. have determined the crystal structure of smallpox topoisomerase protein bound to DNA, where the complex is trapped in an intermediate state of the reaction. The structure provides a number of insights into the topoisomerase reaction, including the way that the enzyme holds onto the ends of the DNA during relaxation of supercoiling and the mechanism of catalysis by conserved amino acids in and near the active site of the enzyme.

E2∼Ub Conjugates Spiraling to Infinity and Beyond

PAGE 138

A key question in ubiquitination is how E2-E3 complex can deal with the various acceptor sites distributed on the substrate surface. The article by Sakata et al. describes the structure of an intermediate of E2∼ubiquitin conjugate which is assembled into an infinite spiral through the noncovalent backside interaction. These findings, along with their biochemical data, underline the significance of a "riding-on" mechanism, in which the self-assembled E2∼Ub conjugates serve as a bridge between the E3 and the acceptor sites, as one of the molecular strategies employed for efficient and versatile ubiquitination of substrates.

